Sensitivity and specificity of HAT Sero-*K*-SeT, a rapid diagnostic test for serodiagnosis of sleeping sickness caused by *Trypanosoma brucei gambiense*: a case-control study

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Summary

Background Human African trypanosomiasis (HAT) is a life-threatening infection affecting rural populations in sub-Saharan Africa. Large-scale population screening by antibody detection with the Card Agglutination Test for Trypanosomiasis (CATT)/*Trypanosoma brucei (T b) gambiense* helped reduce the number of reported cases of gambiense HAT to fewer than 10 000 in 2011. Because low case numbers lead to decreased cost-effectiveness of such active screening, we aimed to assess diagnostic accuracy of a rapid serodiagnostic test (HAT Sero-*K*-SeT) applicable in primary health-care centres.

Methods In our case-control study, we assessed participants older than 11 years who presented for HAT Sero-*K*-SeT and CATT/*T b gambiense* at primary care centres or to mobile teams (and existing patients with confirmed disease status at these centres) in Bandundu Province, DR Congo. We defined cases as patients with trypanosomes that had been identified in lymph node aspirate, blood, or cerebrospinal fluid. During screening, we recruited controls without previous history of HAT or detectable trypanosomes in blood or lymph who resided in the same area as the cases. We assessed diagnostic accuracy of three antibody detection tests for gambiense HAT: HAT Sero-*K*-SeT and CATT/*T b gambiense* (done with venous blood at the primary care centres) and immune trypanolysis (done with plasma at the Institute of Tropical Medicine, Antwerp, Belgium).

Findings Between June 6, 2012, and Feb 25, 2013, we included 134 cases and 356 controls. HAT Sero-*K*-SeT had a sensitivity of 0·985 (132 true positives, 95% CI 0·947–0·996) and a specificity of 0·986 (351 true negatives, 0·968–0·994), which did not differ significantly from CATT/*T b gambiense* (sensitivity 95% CI 0·955, 95% CI 0·906–0·979 [128 true positives] and specificity 0·972, 0·949–0·985 [346 true negatives]) or immune trypanolysis (sensitivity 0·985, 0·947–0·996 [132 true positives] and specificity 0·980, 0·960–0·990 [349 true negatives]).

Interpretation The diagnostic accuracy of HAT Sero-*K*-SeT is adequate for *T b gambiense a*ntibody detection in local health centres and could be used for active screening whenever a cold chain and electricity supply are unavailable and CATT/*T b gambiense* cannot be done.

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Introduction

Human African trypanosomiasis (HAT), or sleeping sickness, is a life-threatening neglected tropical infection affecting rural populations in sub-Saharan Africa.[1] In west and central Africa, the chronic form of sleeping sickness is caused by *Trypanosoma brucei (T b) gambiense*. In eastern and southern Africa, a more fulminant form is caused by *T b rhodesiense*.[2] Infection starts with the bite of an infected tsetse fly (*Glossina* spp). The disease is characterised by two stages, consisting of a haemo-lymphatic stage, with trypanosomes multiplying mainly in lymph and blood, which generally evolves into the second or meningoencephalitic stage in which the central nervous system becomes invaded. If left untreated, the infection is almost invariably fatal.[3] Treatment is possible but present drugs are unsatisfactory because of their toxicity, complex application, and cumbersome logistics.[4,5]

In the 1990s, *T b gambiense* HAT ravaged several west and central African countries. Thanks to the combined efforts of national control programmes, bilateral cooperation agencies, non-governmental organisations, and WHO, fewer than 10 000 new cases were reported in 2011 and elimination of HAT as a public health problem is now regarded as possible.[6,7] One of the key factors leading to this success in HAT control was the adoption of a serodiagnostic test, the Card Agglutination Test for Trypanosomiasis (CATT/*T b gambiense*[8]) as a screening technique. CATT is a rapid serological test that allows screening of large populations for trypanosome-specific antibodies. Only those tests with a positive CATT result need then be subjected to time-consuming

parasitological examinations. However, the CATT test, which is distributed as 50 tests per vial, is designed for mass screening by specialised mobile teams and necessitates a cold chain for proper storage. It is therefore not appropriate for decentralised implementation in peripheral health facilities with few participants to screen on a daily basis, since opened vials have to be discarded after 24 h at ambient temperature and cold chain storage at 4°C is often not available because of intermittent electrical supply in HAT-endemic regions. With the steadily decreasing prevalence of HAT, cost-effectiveness of active screening has diminished, and a subsequent reduction of budgets for screening of the population at risk has meant that not only has the role of the fixed health-care centres for diagnosis, care, and surveillance of HAT become more prominent, but also that the diagnostic techniques need to be adapted. Development of a highly specific and sensitive individual rapid diagnostic test that is stable at ambient temperature and can be used after minimal training is therefore a research priority.[2]

We have previously reported on the development and proof-of-principle of a new rapid serodiagnostic test for gambiense sleeping sickness, called HAT Sero-*K*-SeT.[9] In this study, we aimed to assess the diagnostic accuracy of the HAT Sero-*K*-SeT on clinical samples from well characterised cases and endemic controls in DR Congo by comparison with an existing antibody detection test used in routine screening (CATT whole blood) and a sophisticated antibody assay used in reference laboratories (immune trypanolysis).

Methods

Study design and participants

Our case-control study was done in Bandundu Province, DR Congo. In 2011, the national control programme (Programme National de Lutte contre la Trypanosomiase Humaine Africaine [PNLTHA]) reported an overall prevalence of HAT of 0·18% in this province.[10] Participants were included in the HAT treatment centres of the cities of Masi Manimba, Kikwit, Masa Muna, Nvunda, and Kisalaboyi and during mobile team active screening campaigns in two health districts, Masi Manimba and Bandjow Moke.

All individuals older than 11 years who presented for HAT screening at one of these centres or mobile team sessions, and existing patients with known confirmed disease admitted to the treatment centres, were eligible for study enrolment. All cases were examined in the same way. The definition of a case in this study was a patient with trypanosomes in lymph, blood, or cerebrospinal fluid, irrespective of disease stage. We defined controls as individuals without previous history of HAT or detectable trypanosomes in blood and lymph, who were residing in the same area as the cases. Controls were people who presented for screening (not necessarily relatives or age-matched or sex matched) but in whom no parasites were detected. We excluded participants who provided less than 4 mL of blood for analysis.

The study received clearance from the Ethics Committees of the University of Antwerp, Belgium (11.43.5.795) and of the Ministry of Health, DR Congo (NGCOETH./002/2012). Participants or guardians provided written informed consent before enrolment. We obtained assent from children aged 12–18 years.

Procedures

The reference standard was confirmed presence of parasites detected through the combination of mini Anion Exchange Centrifugation Technique (mAECT) on whole blood and direct microscopic examination of lymph node aspirate and cerebrospinal fluid. Identification of trypanosomes in one test was sufficient to establish diagnosis of confirmed HAT. All consenting participants were first screened with the CATT/*T b gambiense* test (on blood taken from a finger-prick test) and by cervical lymph node palpation, according to the national guidelines issued by the PNLTHA. Irrespective of the CATT whole blood result, 4 mL of venous blood was collected in heparinised tubes for parasitological examination in the mAECT.[11] The remaining blood was used for testing in the HAT Sero-*K*-SeT and for preparation of plasma to be tested in immune trypanolysis. No direct microscopy was done. Lymph aspirate was collected and microscopically examined for trypanosomes from consenting participants who presented swollen cervical lymph nodes. Unless clinically contraindicated, patients with confirmed HAT (ie, those for whom trypanosomes were observed in the lymph node aspirate, blood, or both) underwent lumbar puncture to assess the disease stage before treatment was started. The lumbar puncture was also done, according to national guidelines, on some suspected cases without detectable parasites in blood and lymph but with clear serological and neurological evidence of HAT. For disease staging, we regarded 0–5 white blood cells per µL and no trypanosomes in cerebrospinal fluid as the first stage, and more than 5 white blood cells per µL with or without trypanosomes in cerebrospinal fluid as the second stage. Microscopy was done by experienced personnel, and presence of trypanosomes was always confirmed by a second microscopist. Because identification of trypanosomes in one test is sufficient to establish diagnosis of confirmed HAT, a few patients with HAT did not undergo all parasitological examinations (mAECT or lymph node fluid examination). Treatment was provided according to the national guidelines without reference to results obtained in the HAT Sero-*K*-SeT or immune trypanolysis testing.

HAT Sero-*K*-SeT (Coris BioConcept, Gembloux, Belgium) detects antibodies and can be done with plasma or whole blood. Blood cells are filtered on the sample pad and only plasma migrates over the nitrocellulose membrane while reacting with the target antigens, in this case an equimolar mixture of *T b gambiense* variant

surface glycoproteins LiTat 1.3 and LiTat 1.5, and the conjugate. For this study, 30 µL of blood was dispensed in the sample application window followed by 85 µL of migration buffer (figure). After 15 min, the test result was read as positive if both the control and the test line were visible (even if very faint), negative if only the control line was visible, or invalid if the control line was not visible (in which case a new test was done). Each test was read by two independent readers who were masked to the microscopy results apart from those for patients with confirmed HAT, who were included at the treatment centres while waiting for the start of their treatment. At the start of the study, the test developers (PB and QG) did this reading during 2 weeks, which was subsequently continued by local health staff (trained by PB and QG).

All plasma specimens were preserved in liquid nitrogen for transfer to the Institut National de Recherche Biomédicale in Kinshasa and then to the Institute of Tropical Medicine in Antwerp, Belgium to be tested in immune trypanolysis as the reference test for specific antibodies against *T b gambiense* VAT LiTat 1.3 and LiTat 1.5.[12] Microscopic reading of the results of immune trypanolysis was done by experienced staff, who were masked to other test results.

Statistical analysis

We calculated a sample size of 200 HAT cases would be needed to estimate the sensitivity of the HAT Sero-*K*-SeT within 5% and assuming a sensitivity of at least 85%. Conversely, we calculated a sample size of 350 controls would be needed to show a higher specificity of HAT Sero-*K*-SeT than of CATT while expecting a specificity of 95% for HAT Sero-*K*-SeT and 90% for CATT. However, enrolment had to be stopped prematurely because the accrual rate of cases was much slower than was expected. All results were entered in an Excel database. We calculated sensitivities and specificities with 95% binomial Wilson confidence intervals with Stata 10. We used the McNemar χ^2 test in Stata 10 to test differences in sensitivity and specificity of the HAT Sero-*K*-SeT compared with CATT/*T b gambiense* and immune trypanolysis.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We included participants between June 6, 2012, and Feb 25, 2013. We initially included 135 patients with HAT, although one participant was excluded from analysis because of missing parasitological results in the database. Thus, we included 134 confirmed cases in the analysis (58 [43%] male and 76 [57%] female), with a median age of 33·0 years (IQR 24·5–48·0). 64 patients with HAT were included at the treatment centres while waiting for the start of their treatment. 56 (42%) patients with HAT presented swollen cervical lymph nodes, with confirmed parasite presence in 42 (84%) of 50 participants who had their lymph node fluid tested for presence of parasites. Trypanosomes were detected on blood microscopy in 88 (83%) of 106 patients with HAT. 131 participants had lumbar punctures (three patients had no lumbar puncture for medical reasons such as pregnancy). We noted trypanosomes in the cerebrospinal fluid of 51 (42%) of 122 patients (nine patients had missing parasitological cerebrospinal fluid results in database). 14 patients with positive cerebrospinal fluid samples were negative for trypanosomes in blood and lymph analyses. 39 (32%) of 122 cases (nine had insufficient

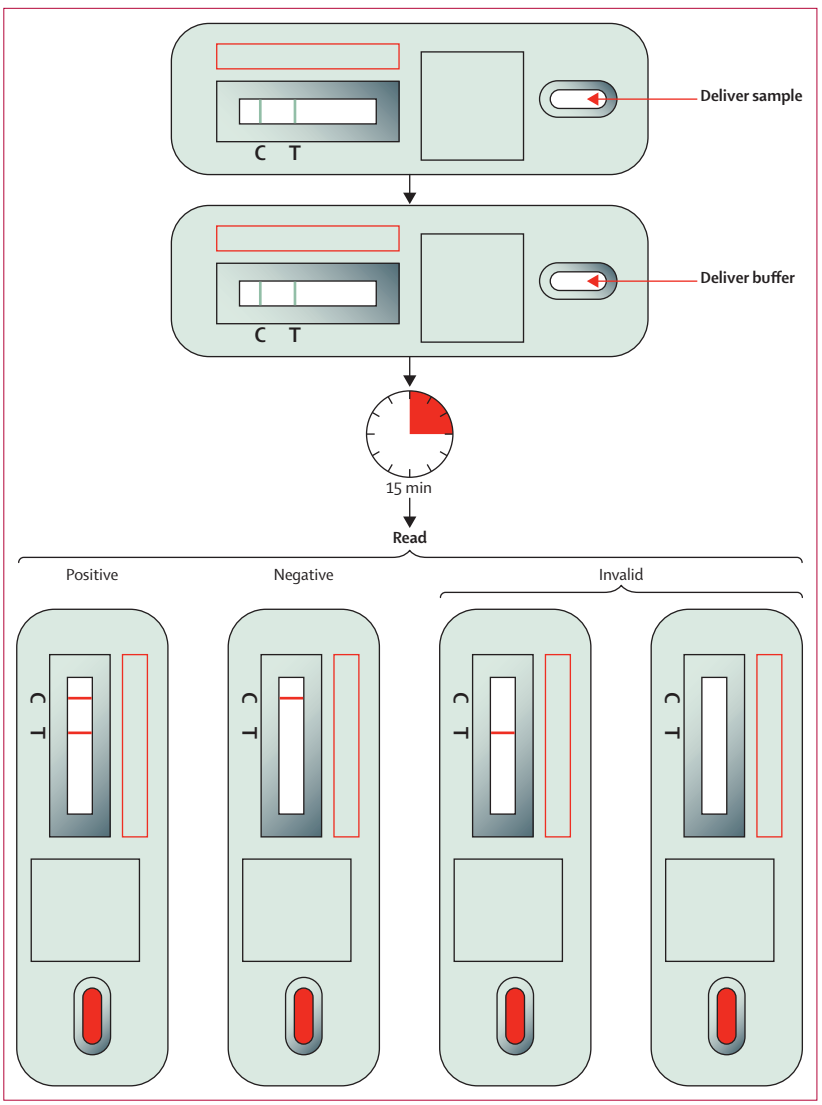

Figure: **HAT Sero-*K*-SeT procedure**
HAT=human African trypanosomiasis.

	Number of patients testing positive	Sensitivity	Number of controls testing negative	Specificity
HAT Sero-*K*-SeT	132/134	0·985 (95% CI 0·947–0·996)	351/356	0·986 (95% CI 0·968–0·994)
CATT whole blood	128/134	0·955 (95% CI 0·906–0·979)	346/356	0·972 (95% CI 0·949–0·985)
Immune trypanolysis	132/134	0·985 (95% CI 0·947–0·996)	349/356	0·980 (95% CI 0·960–0·990)

No missing or indeterminate results were noted for any test. CATT=Card Agglutination Test for Trypanosomosis. HAT=Human African trypanosomiasis.

Table: **Sensitivity and specificity of the three serological tests, with combined parasitology as a reference**

results for staging in database) were in the first stage of the disease. We noted microfilaria in two cases on mAECT.

We included 363 participants as non-HAT controls, of whom seven were excluded from the analysis because of inconsistent or missing results from the database (four participants) or no plasma available for immune trypanolysis test (three participants). Thus, we included 356 controls in the analysis (144 [40%] male and 212 [60%] female, with a median age of 29·0 years (IQR 21·0–42·0). We noted microfilariae in the blood of 16 controls.

We had no discordant readings with HAT Sero-*K*-SeT between the two independent readers. On one occasion, the test line was scored as very faint by the two observers and the opinion of a third masked reader was sought, who confirmed the faint test line as positive. The table shows sensitivity and specificity of the three serological tests.

We noted no significant difference in sensitivities or specificities between HAT Sero-*K*-SeT versus CATT whole blood (p=0·16 for sensitivity and p=0·06 for specificity), CATT versus immune trypanolysis HAT (p=0·16 for sensitivity and p=0·44 for specificity), or HAT Sero-*K*-SeT versus immune trypanolysis (p=1·00 for sensitivity and p=0·53 for specificity). Of seven controls with positive results on immune trypanolysis (2%), only one tested seropositive in the field, on both CATT whole blood and in HAT Sero-*K*-SeT analyses.

Panel: **Research in context**

Systematic review

We did not do a systematic review before this trial was started. Three rapid diagnostic tests for serodiagnosis of human African trypanosomiasis (HAT) have been described,[5,14] but to our knowledge no phase 2 diagnostic trial of these tests has been reported in peer-reviewed journals.

Interpretation

Our trial showed the diagnostic accuracy of HAT Sero-*K*-SeT, an individual rapid serodiagnostic test for HAT in a case-control study done in tropical field conditions in DR Congo (the country that reports most cases of the infection). The results suggest that the choice of a screening test for this disease can be based on the test format, because diagnostic accuracy of HAT Sero-*K*-SeT was not different from other test formats that are more suitable for mass screening and for reference testing. HAT Sero-*K*-SeT facilitates detection at peripheral health facilities and might contribute to elimination of HAT.

Discussion

The high sensitivity (98·5%) and specificity (98·6%) we noted for HAT Sero-*K*-SeT on venous blood samples supports the level of diagnostic accuracy reported in a phase 1 trial with reconstituted blood samples and are much the same as reports for another rapid diagnostic test for gambiense sleeping sickness, the HAT SD Bioline.[9,13] HAT Sero-*K*-SeT, CATT whole blood, and immune trypanolysis are very similar with regard to the antigens presented, because they are all based on native variant surface glycoprotein, with a combination of LiTat 1.3 and LiTat 1.5 for HAT Sero-*K*-SeT and immune trypanolysis and LiTat 1.3 only for CATT. In theory, the use of a single variant surface glycoprotein in CATT might lead to a lower sensitivity, which might be counterbalanced by exposure of other non-variant epitopes on the fixed reagent, which in turn could affect specificity. Patients infected with trypanosomes that do not express these variant surface glycoproteins might not develop antibodies against the antigens in use, and remain negative in all three tests, but such patients are probably rare. Nevertheless, the diagnostic ability of all three serological tests was excellent in our assessment and did not differ significantly between groups.

Our study had limitations. To be able to enrol sufficient participants, about half of the cases were recruited in the treatment centres to which they were referred by a mobile team on the basis of an initially positive CATT/*T b gambiense* result. This strategy might have made us overestimate the sensitivity of the tests because it could have caused a selection bias towards the LiTat 1.3 antigen in cases. Moreover, because test readers were not masked to the microscopy results of these patients and although both readings were done independently, theoretically this approach could have led to improved rates of reading, especially for very faint test lines. The restricted geographical distribution of the participants in this study might be regarded as another limitation. However, this study site lies about 1000 km away from the HAT foci in East Kasai Province, DR Congo, from where the specimens used in the phase 1 assessment of the HAT Sero-*K*-SeT originated.[9] We used a composite reference standard in this study, based on combined parasite detection results. Although this technique

increases the sensitivity of the reference standard, we acknowledge it might not reach 100%. Therefore, our control group might have contained some positive (but undetected) cases of HAT, resulting in a relative underestimation of the serological test specificity.

Our results suggest that choice of a screening test for HAT can be based on the format that fits the purpose best. For example, CATT/*T b gambiense* seems most suitable for active case detection (ie, large-scale screening) with mobile teams that have access to electricity from a generator, a car battery, or a solar panel, whereas HAT Sero-*K*-SeT seems well adapted for use in peripheral health centres (ie, centres with a low number of suspected cases and no or intermittent electricity supply) or for active case detection in less accessible locations when CATT/*T b gambiense* cannot be done for logistical reasons (panel). Immune trypanolysis requires more sophisticated equipment and skills, and its use is therefore restricted to centrally located laboratories, which can use the test to process large batches of samples from epidemiological surveys or surveillance activities.

The next step in the development of the HAT Sero-*K*-SeT will be assessment of diagnostic accuracy in a prospective study on a series of patients presenting at health centres with suspected HAT. Such a phase 3 diagnostic trial is already ongoing in DR Congo. Rapid diagnostic tests such as HAT Sero-*K*-SeT offer an opportunity to simplify disease detection at the peripheral health facilities and will become valuable methods to reach the goal of HAT elimination.[15]

Contributors

PB, PM, TL, and VL designed the study. PB, QG, DM-N, and PPP did the fieldwork. DJ did the trypanolysis assay. PB and QG collected the data. VL and MB analysed the data. All authors contributed to the writing of the report.

Declaration of interests

PM, QG, and TL are employees of Coris BioConcept. All other authors no competing interests.

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